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The effect of ATP, GTP and cAMP on the cGMP-dependent conductance of the fragments from frog rod plasma membrane

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Using a 'patch-clamp' method in the 'inside-out' configuration, ATP, ADP, AMP-PCP and AMP-PNP have been shown to increase the cGMP-dependent component of the rod plasma membrane conductance 2–4-fold and GTP, GDP but not GMP or nonhydrolyzable GTP analogs GMP-PNP and GTP-γ-S to abolish the ATP action. The ATP and GTP effects were observed at [EDTA] = 1 mM when magnesium and calcium ions were absent. In about half of the experiments the cGMP-dependent conductance was shown to be increased by cAMP in the micromolar concentration range by 10–50%, the cAMP action did not depend on the presence of nucleoside triphosphates. In vivo ATP, GTP and cAMP are assumed to modulate the sensitivity of the photoreceptor plasma membrane to cGMP.

Retinal rod; cyclic GMP; Cationic channel; Conductance regulation

1. INTRODUCTION

As it has been shown earlier, cyclic GMP activates the cationic conductivity of the fragments of the retinal rod outer segment plasma membrane [1–5]. Recently Matthews and Watanabe [6] have unequivocally demonstrated the identity of the cyclic GMP-activated and light-dependent channels. The data represented in this paper show that the cGMP effect on the rod membrane can be modulated by ATP, GTP, cAMP, ADP and GDP.

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Abbreviations: ADP, adenosine-5′-diphosphate; AMP, adenosine-5′-monophosphate; AMP-PCP, adenylyl (β,γ-methylene)diphosphonate; AMP-PNP, adenylyl-imidodiphosphate; cAMP, adenosine-3′,5′-monophosphate, cyclic; ATP, adenosine-5′-triphosphate; 8BrcGMP, 8-bromo-guanosine-3′,5′-monophosphate, cyclic; CTP, cytidine-5′-triphosphate; GDP, guanosine-5′-diphosphate; GMP, guanosine-5′-monophosphate; GMP-PNP, guanylyl-imidodiphosphate; cGMP, guanosine-3′,5′-monophosphate, cyclic; GTP, guanosine-5′-triphosphate; GTP-γ-S, guanosine-5′-*O*-(3-thiotriphosphate); EC_{50}, concentration of an agent at which its effect reaches 50% of maximal value; Hepes, 4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid; ROS, rod outer segment; UTP, uridine-5′-triphosphate

2. MATERIALS AND METHODS

The methods used to obtain gigaseals and those for measuring the electric parameters of the isolated 'inside-out' patches were identical to those described earlier [1,2,7]. The experiments were carried out with the rods from *Rana temporaria* and *Xenopus laevis*.

In this work we used 8BrcGMP, AMP-PCP, AMP-PNP, GMP-PNP, GTP-γ-S, Hepes from Boehringer (Austria); ADP, ATP, GTP, AMP, GDP, GMP, UTP, CTP and cAMP from Reanal (Hungary); EDTA from Serva (FRG); other chemicals used were of chemical grade without additional purification from Reachim (USSR). If not otherwise mentioned, we have used saline of the following composition (mM): 100 NaCl, 2.5 KCl, 2 $MgCl_2$, 0.1 $CaCl_2$, 10 Hepes, pH adjusted to 7.5 NaOH.

3. RESULTS

3.1. *The effect of nucleoside tri- and diphosphates and their analogs on the cyclonucleotide-dependent component of the rod plasma membrane conductance*

In all the experiments ATP used within the 0.5–1 mM range increased the cGMP-dependent component of the conductance. The action of ATP

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was reversible and could be observed repeatedly.

In the absence of cyclonucleotide, the changes in the patch conductance when ATP was applied did not exceed 10–15% of the changes of the cyclonucleotide-dependent component and were not studied.

We observed three types of dependences of the cGMP-dependent conductance on ATP concentration.

Type 1 was characterized by a bell-shaped dependence (4 cases, curve 1 in fig.1a) with a maximum at 0.5–1 mM and an EC_{50} value for the rising phase of about 0.1–0.3 mM. At high ATP concentrations (several mmol/l) the cGMP-dependent conductance was suppressed to a level lower than that of the control (without ATP). The dependence of type 2 (3 cases, curve 2 in fig.1A) possessed a monotonous character without inflexion points in the range 0–5 mM. In three cases a type 3 dependence (a monotonous one with an inflexion point) was observed. It was interpreted as a superposition of the dependences of types 1 and 2 (fig.1C).

ADP and nonhydrolyzable analogs of ATP, AMP-PNP and ADP-PCP, also increased the cGMP-dependent conductance although to a lesser degree than ATP (fig.1C). AMP was quite ineffective. When, using one and the same patch, it was possible to obtain the dependences of cGMP-activated conductance upon the concentration of ATP and its nonhydrolyzable analog, these dependences were similar (fig.1C). The action of ATP did not depend on the presence of divalent cations because it was observed when standard saline was exchanged for a similar one containing EDTA (1 mM) but not magnesium and calcium salts.

In most of the experiments the effect of GTP on the cGMP-dependent conductance was essentially weaker than that of ATP. The experiments, the results of which are given in this paper, were carried out on such patches. Some patches showed a substantially higher sensitivity to GTP; the GTP action on them was similar to that of ATP. The effect of high sensitivity to GTP does not lie within the scope of this paper. At present this problem is still being studied.

3.2. *GTP-ATP antagonism*

As mentioned above, the action of GTP on the

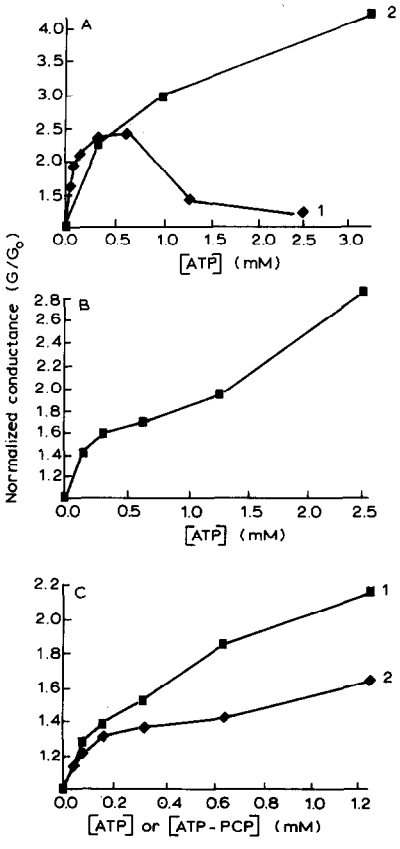

Fig.1. Examples of the dependences of the cGMP-activated conductance, represented in a normalized form, on the concentration of ATP (A,B,C) and ATP-PCP (C). [8BrcGMP] = 5 μM. (A) Curves: 1, the dependence of type 1 (bell-shaped); 2, the dependence of type 2 (monotonous without inflexion points). (B) The dependence of type 3 (possibly a superposition of 1 + 2). (C) The dependences of conductance of one and the same patch on the concentration of ATP (1) and ATP-PCP (2).

cGMP-dependent conductance was far less essential than that of ATP. Furthermore, it turns out that ATP and GTP in combination are considerably weaker than ATP alone (fig.2). The inhibitory action of GTP on the effect of ATP was reversible and could be repeatedly observed on one and the same patch; it did not depend on the ATP concentration (fig.3A,B) and was characterized by an EC_{50} value of ~0.5 mM. Hence, the inhibitory action of GTP on the ATP effect was not caused by their competition for one and the same site.

GDP, but not GMP, GMP-PNP and GTP-γ-S, appeared to possess the capability of suppressing

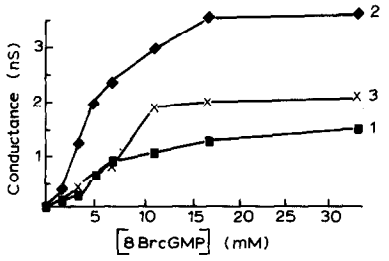

Fig.2. The dependences of conductance of the rod plasma membrane patch on the concentration of 8BrcGMP at different concentrations of ATP and GTP. Curves: 1, [ATP] = [GTP] = 0; 2, [ATP] = 3.3 mM, [GTP] = 0; 3, [ATP] = 3.3 mM; [GTP] = 1 mM.

the ATP action. This capability was comparable with GTP.

Thus, either the GTP and GDP action is due to their hydrolysis or the nonhydrolyzable analogs of GTP do not interact with the corresponding sites.

The GTP action did not depend on the presence of Ca^{2+} and Mg^{2+}. UTP and CTP did not affect the cGMP-dependent conductance.

3.3. *Modulation of the cGMP-dependent conductance by cAMP*

In 8 experiments of 13 it was found that cAMP at micromolar concentrations increased the

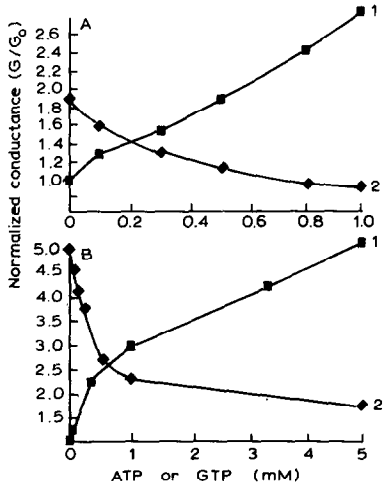

Fig.3. The dependence of the inhibitory action of GTP on the ATP effect on GTP concentration. Curves 1 are the dependences of the cGMP-activated conductance on ATP concentration at [GTP] = 0. Curves 2 are the dependences of cGMP-activated conductance on GTP concentration at [ATP] = 5 mM (A) and [ATP] = 0.5 mM (B). All dependences are given in a normalized form.

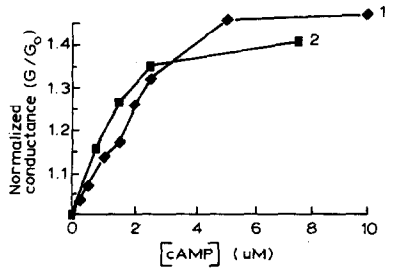

Fig.4. Examples of the dependence of cGMP-dependent conductance of ROS membrane patches on the cAMP concentration. [8BrcGMP] = 7.5 μM (1) and 2 μM (2).

cGMP-dependent conductance (EC_{50} ~ 1–2 μM) by 10–50%, and that the cAMP action did not depend on the presence of ATP or GTP. As a rule, the amplitude of this effect was not large, reaching 40% of the cGMP-dependent conductance magnitude in only two experiments. The dependences of conductance on the cAMP concentration obtained for these patches are shown in fig.4.

4. DISCUSSION

Earlier it was stated that ATP, GTP and cAMP did not affect the ROS plasma membrane patches and that the cGMP effect did not depend on the presence of ATP and GTP [1,2]. As far as cAMP is concerned, it is not difficult to explain such statements: the cAMP action does not always occur and, as a rule, its magnitude is not large. In our opinion, two circumstances should be taken into account when explaining why modulation of the cGMP-dependent conductance by nucleoside triphosphates was not revealed earlier. Firstly, in previous work [1,2] the combination of 1 mM ATP + 1 mM cGMP, being practically ineffective because of the GTP-ATP antagonism, was used in some experiments. Secondly, the dependence of cGMP-activated conductance on the concentration of ATP is often bell-shaped and the conductance level, which differs slightly from that of the control, corresponds to the usual concentration (1 mM) in most experiments.

The effects of ATP and cAMP described in this paper are effected without the participation of nucleoside triphosphate hydrolysis and are conditioned by a direct interaction of the agents with the

membrane components. Either the cGMP-activated channel, which also has ATP, GTP and cAMP-binding sites, or other membrane proteins change their conformation and, therefore, their manner and degree of interaction with the cGMP-dependent channel may serve as such components.

As regards the GTP-ATP antagonism, the enzyme hydrolysis of GTP and GDP may participate in effecting this phenomenon.

At present cGMP is usually believed to be the main or even the only mediator in vision excitation [8,9]. The results of this paper indicate that the conductance of rod plasma membrane may be regulated by at least four compounds. So, it is noteworthy that, firstly, the cAMP pool in photoreceptors is light-dependent [10]; secondly, that cAMP was reported to activate the cationic conductance of intracellularly dialysed ROS within the micromolar concentration range [11]. It is not expected that under certain conditions cAMP may be a co-regulator of the photoreceptor plasma membrane conductance. And what about nucleoside triphosphates, the ATP/GTP ratio alterations could change the membrane conductance. For instance, the substantial decrease in GTP concentration after illumination revealed by Bownds and co-workers [12] may lead to an increase of membrane sensitivity to cGMP which could be one of the mechanisms of light adaptation. Such an alteration of GTP concentration may result not only from hydrolysis by guanylate cyclase and transducin, but also from the functioning of a powerful transphosphorylation system present in ROS. The functions of the system are still unknown [13].

REFERENCES

[1] Fesenko, E.E., Kolesnikov, S.S. and Lyubarsky, A.L. (1985) Nature 313, 310–313.
[2] Fesenko, E.E., Kolesnikov, S.S. and Lyubarsky, A.L. (1986) Biochim. Biophys. Acta 856, 661–672.
[3] Stern, J.H., Kaupp, U.B. and MacLeish, P.R. (1986) Proc. Natl. Acad. Sci. USA 83, 1163–1167.
[4] Zimmerman, A.L. and Baylor, D.A. (1986) Nature 321, 70–72.
[5] Haynes, L. and Yau, K.-W. (1986) Nature 321, 72–74.
[6] Matthews, G. and Watanabe, S.-I. (1987) J. Physiol. 389, 691–715.
[7] Kolesnikov, S.S., Lyubarsky, A.L. and Fesenko, E.E. (1984) Vision Res. 24, 1295–1300.
[8] Stryer, L. (1986) Annu. Rev. Neurosci. 9, 87–119.
[9] Kaupp, U.B. and Koch, K.-W. (1986) Trends Biochem. Sci. 11, 43–47.
[10] Cohen, A.I. (1982) J. Neurochem. 38, 781–796.
[11] Kurkin, S.A., Kislov, A.N. and Fesenko, E.E. (1982) Biofizika 27, 1053–1056.
[12] Biernbaum, M.S. and Bownds, M.D. (1985) J. Gen. Physiol. 85, 107–121.
[13] Schnetkamp, P.P.M. and Daemen, F.J.M. (1981) Biochim. Biophys. Acta 672, 307–312.